UTILIZATIONS OF THE SFECIAL CANKER LESION ON SUNKI LEAVES

by

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INTRODUCTION

Citrus canker caused by *Xanthomonas citri* (Hasse) Dowson is normally characterized by corky, hard, and somewhat suberized lesions surrounded by a yellowish halo on the infected leaves of susceptible varieties. If the canker lesion has developed quickly under extremely favorable conditions, the white spongy tissue will excruded in some degree, and accumulated on the lesions. This special canker lesions with spongy eruption of tissue will be found, sometime, when artificial inoculation was made by overstaying the inoculated plant in pot (Fig. 4) in the inoculation chamber for three or four days after inoculation. It is also easy to induce special lesions by keeping the leaves of susceptible varieties in such a semi-vivo condition as in the moistened Petri-dish (Fig. 3) placed in the incubator for five or six days after inoculation.

The present author was deeply interested in the formation of this special lesion and had made his attempt to utilize them for several purposes, such as screen test for canker-control bactericides, identrfication of newly isolated canker organism, and determination of resistance of citrus varieties to the disease. The present report was made in deal with utilization of this inoculation technique for testing inhibiting effectiveness of the bactericides on the fomation of canker lesion.

MATERIALS AND METHODS

Two pieces of roof-shaped plastics with two cuts on their ridge were placed on the filter paper in the Petri-dish, 12 cm in diameter. Two glass rods were then fitted on the cuts of the plastic supporters to form a bridge-like frame (Fig. 1). Enough water for wetting the filter paper was poured into the Petri-dish.

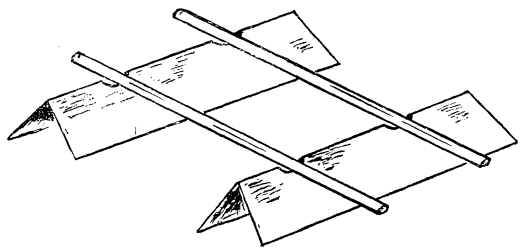


Fig. 1. Bridge-like frame in moistened Petri-dish.

* The author wish to acknowledge his indebtedness to Dr. H. J. Su of National Taiwan University for his valuable suggestions and criticisms during preparation of this paper.

Leaves of Sunki (*Citrus sunki*) seedling grown in the experimental farm of the Taiwan Agricultural Research Institute were detached from the base of the petiole with a scissors and were throughly washed by gentle soaking in the running tap water followed by drying with filter paper.

Definite number of wounds were carefully made by puncturing the leaves on two or three layers of filter paper with dissecting needle. Bacterial suspension was prepared in 100 ml conical flask by dissolving the colony of bacteria cultured on the nutrient agar slant at 28°C for 48 hrs with sterile distilled water.

The Sunki leaves mentioned above were dipped individually in the bacterial suspension for certain minutes and then were placed on the glass frame in the Petri-dish. Keep the Petri-dish in the incubator for six days before reading. The percentage of the lesion developed was calculated by direct counting of the number of lesions.

LESION DEVELOPMENT

In order to clarify the relation between the maturity of the leaves and the development of the special lesion, the leaves were divided into four grades of maturity as young, premature, mature and old, according to their size and color. Premature leaves were fully expanded but still yellowish green in color, and mature leaves were dark in color. The lesion most easily developed on these leaves according to the resultant data. Young, not fully extend, and old leaves, nearby falling, however, easily either corrupted or dried out during the experiment (Fig. 6).

As for the temperature in relation to the development of the lesion, 28°C was most favorable for the lesion development, although 22°C, 25°C, 31°C and 34°C were also favorable as suggested by the resultant data. Horever, no lesion developed at 16°C. The leaves frequently dried out at 37°C, although a few lesions were found at this temperature.

The size of the wound is important to the development of the lesions, for most of them were formed more distinctly on the wound on which no necrotic were examined. Accordingly, it is necessary to pay the special carefulness and use a fine needle during the puncturing in order to make a nice wound.

SCREEN TEST OF THE FUNGICIDES FOR THE DISEASE CONTROL

The chemicals and their active ingredients used in this experiment are shown in Table 1.

The stock solution of each fungicide was prepared saparately with sterile distilled water in 125 ml conical flask. Dilution was made by transferring each stock solution in the flask with a pippet into 100 ml beaker and enough dose of sterile water was added up to a given concentration. The leaves punctured, fourty wounds a leaf, were inoculated by soaking them in the bacterial suspension in 1000 ml beaker for 5 minutes followed by dipping in the fungicide solution for 10

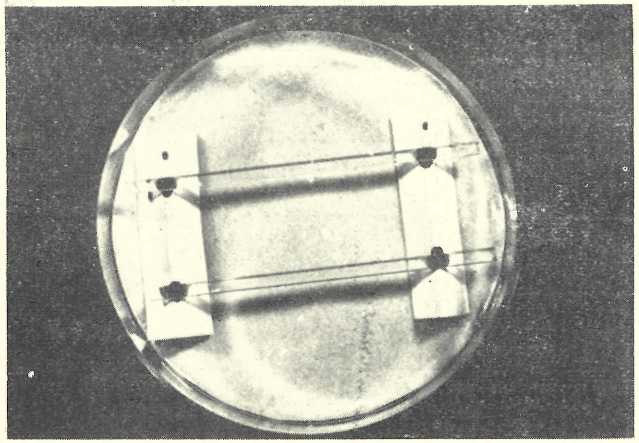


Fig. 3. Moistened Petri-dish for the special canker lesion formation.

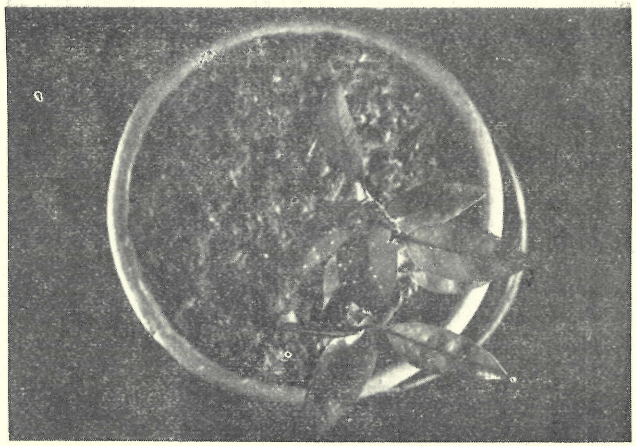


Fig. 4. The special lesions on the leaves of inoculated Sunki seedling in pot.

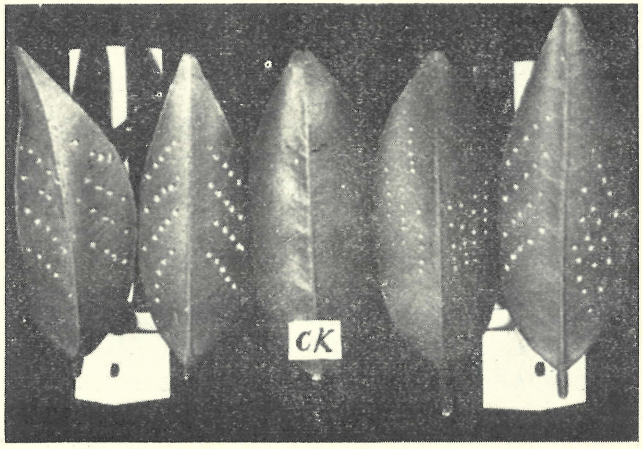
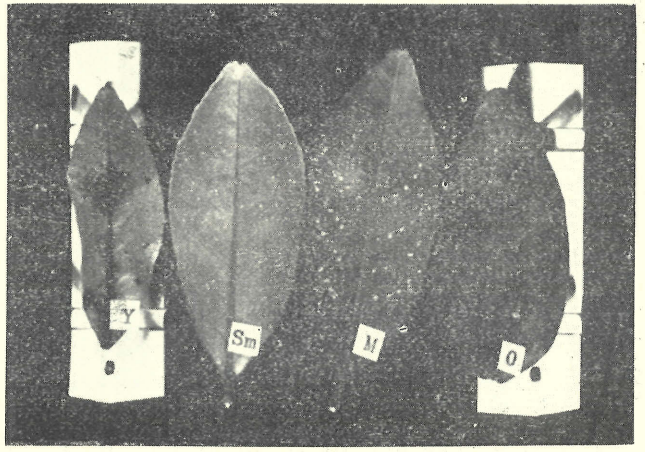


Fig. 5. The special canker lesions formed on detached leaves of *Citrus sunki*.



Fig, 6. Showing the special canker lesions formed on Sunpi leaves of different maturities.

Table 1. Chemicals and their active ingredients used

Chemicals	Active ingredients	Manufecturer
Agrimycin-100	Streptomycin, 15% Terramycin 1.5%	Pfizer Inc., U. S. A.
"Ortho" streptomycin	Streptomycin sulfate 21.2%	Califonia Spray- Chemical Corp., U. S. A.
"Takeda" mycin	Dihydrostreptomycin 2.5%	Takeda K. K., Japan
"Nichinou" phytomycin	Streptomycin 4.5%	Nihon Nohyaku K. K., Japan
"Sankyo" phytomycin	Streptomycin 4.5%	Sankyo K. K., Japan

minutes. The dropdews on the leaves were dried with filter paper. Each four leaves were placed in the Petri-dish and incubated.

Incubation at 28°C was ended six days after inoculation. The resultant dada are shown in Table 2. The regression lines were calculated and plotted on the graph paper (Fig. 2).

Table 2. Inhibitory effect of the chemicals on the development of canker lesions on Sunki leaves

Conc.	Agrimycin-100		Ortho Streptomycin		Takedamycin		Sankyo Phytomycin		Nichinou Phytomycin		CK	
(ppm)	I.*	D.**	I.	D.	I.	D.	I.	D.	I.	D.	I.	D.
12.5	7.0	93.0	5.5	94.5	7.5	92.5	2.5	97.5	10.0	90.0	0.0	100
	11.5	88.5	9.0	91.0	0.0	100.0	7.5	92.5	14.0	86.6	0.0	100
	5.0	95.0	0.0	100.0	15.5	84.5	0.0	100.0	0.0	100.0	0.0	100
25	17.5	82.5	7.0	93.0	0.0	100.0	6.5	93.5	24.0	76.0		
	28.5	71.5	23.0	77.0	41.5	58.5	10.0	90.0	8.5	91.5		
	19.5	80.5	0.0	100.0	10.5	89.5	0.5	99.5	0.5	99.5		
50	15.5	84.5	26.5	73.5	5.0	95.0	12.0	88.0	11.5	88.5		
	32.5	67.5	6.0	94.0	39.5	60.5	35.5	64.5	40.0	60.0		
	37.0	63.0	13.5	86.5	15.0	85.0	28.5	71.5	1.5	98.5		
100	77.5	22.5	0.0	100.0	20.0	79.5	5.5	94.5	6.5	93.5		
	59.5	40.5	40.0	60.0	36.5	63.5	29.5	70.5	53.0	47.0		
	25.0	75.0	36.0	64.0	12.5	87.5	58.0	42.0	3.5	96.5		
200	80.0	20.0	47.5	52.5	25.0	75.0	25.0	75.0	40.0	60.0		
	63.5	36.5	13.5	86.5	37.5	63.0	27.5	72.5	59.0	41.0		
	38.5	61.5	57.5	42.5	30.5	69.5	55.5	44.5	19.5	80.5		

* Percentage of lesion inhibited

** Percentage of lesion developed

Culculation of regression line:

1. Agrimycin-100:

$$byx=\frac{n\Sigma xy-\Sigma x\Sigma y}{n\Sigma x^2-(\Sigma x)^2}=\frac{15(58781.25)-(1162.5)(518)}{15(159843.75)-(1162.5)^2}=0.27$$

$\Sigma y=na+byx\cdot\Sigma x$ $\quad a=13.61$ $\quad y=13.61+0.27x$

$$\text{Correlation coefficient } (r)=\frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2\cdot\Sigma(y-\bar{y})^2}}=\frac{18636.25}{24446.56}=0.76$$

$$\text{F value}=\frac{r^2\Sigma(y-\bar{y})^2/1}{(1-r)^2\Sigma(y-\bar{y})^2/n-2}=\frac{r^2(n-2)}{1-r^2}=\frac{7.5088}{0.4224}=17.78$$

$17.78>F_{0.05}=4.67$

2. "Ortho" streptomycin:
   byx=0.18 a=5.05 y=5.05+0.18x
   r=0.68 $F=11.18>F_{0.05}=4.67$
3. "Takeda" mycin:
   byx=0.10 a=12.02 y=12.02+0.1x
   r=0.49 $F=4.11<F_{0.05}=4.67$
4. "Sankyo" phytomycin:
   byx=0.17 a=7.09 y=7.09+0.17x
   r=0.67 $F=10.59>_{0.05}=4.67$
5. "Nichinou" phytomycin:
   byx=0.16 a=7.03 y=7.03+0.16x
   r=0.58 $F=6.58>F_{0.05}=4.67$

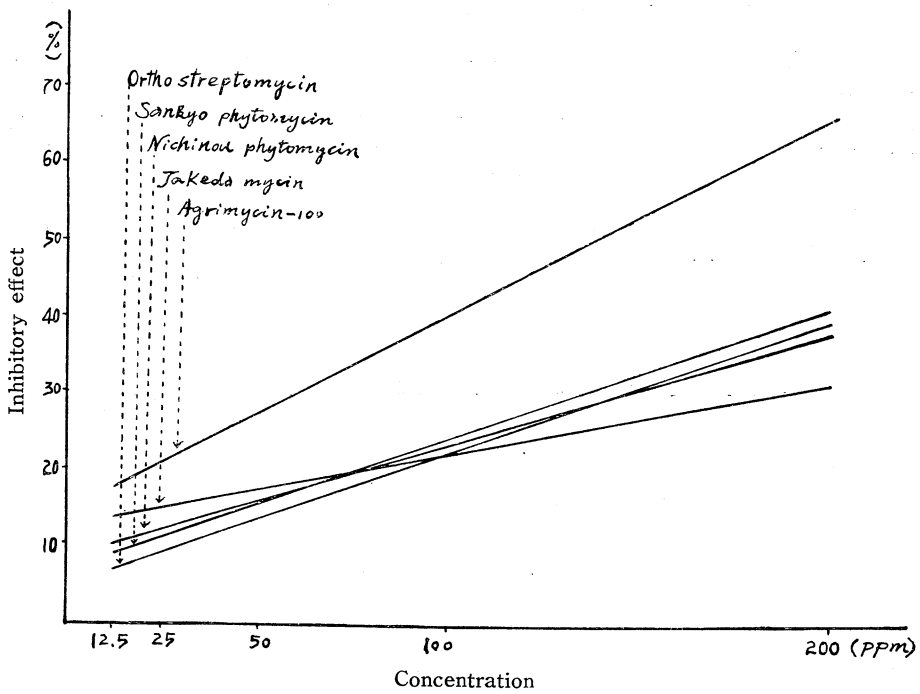


Fig. 2. DR curves of different bactericides at various concentrations.

DISCUSSION AND CONCLUSION

Like other pathogenic bacteria, the canker bacterium, *Xanthomonas citri* is killed in vivo by bactericides at higher concentration than in vitro. Some troubles were frequently borthering the investegators in their screen tests on bactericiides for disease control because of the different results obtained in laboratory and field test.

It was experienced that the distinctive special canker lesion was easily formed on Sunki leaves by artificial inoculation under extremely favorable conditions. The author has tried to employ this inoculation technique for screen test of the canker-control bactericides in his laboratory. It is easy to obtain Sunki leaves since the citrus variety has been widely used as root-stock on this Island. The present method designed by the author seems to be easily conduted and just the

simple facilities are necessary during the experiment. Moreover, it takes brief in time for carying out the experiment.

The significant regression line showing the correlation between the formation of the special lesions and inhibition by some test bactericides at various concentrations was obtained as shown in the resultant data mentioned above. It is recognized by the author that this methed might be a convenient and recomendable way for preliminary experimant for screen test with bactericides for canker control.

LITERATURE

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酸桔葉片殊形潰瘍病斑之利用

楊　騰　金

摘　要

柑桔類潰瘍病於極度適宜之環境下所發生之病斑，因罹病組織之海棉細胞受病原菌之刺激致呈畸形膨大，多量溢出，而形成白色隆起之明顯殊形病斑，此種殊形病斑頗易由人工接種而顯現。將酸桔半成熟葉片採下，以自來水冲洗後以解剖針輕刺一定數目之傷痍，浸漬於病原菌懸浮液，使葉面餘液陰乾後排放於潮濕培養皿之玻璃支架上，置 28°C 定溫箱培養六天，卽可於傷痍部位呈現明顯之殊形潰瘍病斑。

筆者以此病斑形成之明顯與容易，乃探測其利用價值。本試驗以五種抗生製劑供作傷痍葉片人工接種後作藥劑浸漬處理，以葉片病斑發生數目，測定供試藥劑對病原菌之抑制或殺滅效力。據試驗結果，病斑之發生與藥劑濃度具有規律之相關關係。筆者認爲此法於藥劑試驗之初步選定上，爲一簡便而可行之方法。